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TITLE: Development of a Novel Alginate-Based Pleural Sealant

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

A variety of lung diseases such as emphysema, infections, and lung cancers as well as lung injury from trauma, including battlefield trauma, and complications of respirator life support of critically ill patients in intensive care units can result in lung collapse that can be immediately life-threatening or result in chronic leaking of air or fluid out of the lung. These remain challenging medical problems for which few good options are currently available and result in significant morbidity, mortality, hospital stays, health care costs, and other complications. New options are thus desperately needed. We are developing a novel approach to provide an easy-to-apply lung sealant which can repair lung leaks. This initially involved use of a chemically modified form of alginate, a naturally occurring seaweed derivative, increasingly being explored for a variety of biomedical applications. Particular attributes include easy availability, low cost, easy use, biodegradability, and lack of significant toxicity. In the studies to date, we have done extensive materials characterization not just of modified alginates but now a number of other biologic compounds that also have potential as pleural sealants. We have further extensively evaluated promising compounds using small (rodent) and large (pig) ex vivo lung models and have performed initial in vivo evaluations of several compounds in a non-survival surgery rat lung injury model. The studies to date have thus identified several promising compounds that will be further evaluated in the non-survival surgery and also a survival surgery rat lung injury model during the 6 month extension period of the grant. These will lead to a firm platform for further investigations in large animal survival surgery models and subsequent discussions with the FDA about new IND for a clinical investigation.

15. SUBJECT TERMS
Lung, lung health, lung disease, pneumothorax, pleura, pleural sealant, alginate

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1. Introduction

Lung injury is a commonly encountered pathology that can be immediately life-threatening or result in the chronic leakage of air or fluid out of the lung, causing significant morbidity for patients and cost to the healthcare system. Lung injury can stem from etiologies as diverse as COPD, infection, cancer, trauma – including battlefield trauma, and prolonged mechanical ventilation in critically ill patients among others. These remain challenging medical problems for which few good options are currently available and result in significant morbidity, mortality, hospital stays, health care costs, and other complications. New options are thus desperately needed. We are developing a novel approach to provide an easy-to-apply lung sealant which can repair lung leaks. This initially involved use of a chemically modified form of alginate, a naturally occurring seaweed derivative, increasingly being explored for a variety of biomedical applications. Particular attributes include easy availability, low cost, easy use, biodegradability, and lack of significant toxicity. In the studies to date, we have done extensive materials characterization not just of modified alginates but of several other biologic compounds that also have potential as pleural sealants. We have extensively evaluated promising compounds using small (rodent) and large (pig) *ex vivo* lung models and have performed initial *in vivo* evaluations of several compounds in a non-survival rat lung injury model. These results have lead us into initial cohorts of survival surgeries in rat lung injury models. Promising compounds will be further evaluated in the survival surgery rat lung injury model. This will lead to a firm platform for further investigations in large animal survival surgery models and subsequent discussions with the FDA about new IND for a clinical investigation. To this end, we have submitted a letter of intent to the DOD for a Technology/Therapeutics Development Award proposal to be submitted this Fall to to continue the relevant pre-clinical studies.

2. Keywords

Lung, lung health, lung disease, pneumothorax, pleura, pleural sealant, alginate

3. Accomplishments

a) What were the major goals of the project? Listed from the Statement of Work

Specific Aim 1(specified in proposal) **To optimize the modified alginate for use as a pleural sealant**
Major Task 1: Develop chemically modified alginate (AA-MA) hydrogels and characterize material properties.
Subtask 1: Synthesize and chemically characterize AA-MA polymer formulations.
Subtask 2: Quantify the viscosity and shear mechanical properties of AA-MA solutions and hydrogels.
Milestone(s) Achieved: An elastic AA-MA hydrogel will be fabricated with controllable degrees of methacrylation and crosslinking.

Major Task 2: Assess the burst pressure strength and adhesiveness of AA-MA hydrogel sealants.
Subtask 1: Measure burst pressure and analyze cohesion and adhesion of AA-MA hydrogels on collagen substrates.
Subtask 2: Synthesize AA-MA hydrogels with the ability to covalently link to tissue proteins or create cell-material linkages.
Milestone(s) Achieved: AA-MA hydrogel sealant will exhibit burst pressures beyond the physiological range and will remain adhered to underlying substrate/tissue up to burst pressure.

Specific Aim 2(specified in proposal) **To assess the use of optimized modified alginates in an in vivo rat lung injury model**
Major Task 1: Assess different modified alginate hydrogels and patches in an open-chest in vivo rat model.
Subtask 1: Assess different alginate formulations in the non-survival rat surgery model: evaluation of lung mechanics.
Subtask 2: Assess different alginate formulations in the non-survival rat surgery model: histologic evaluation of lung tissues.
Milestone(s) Achieved: An elastic AA-MA hydrogel will be fabricated which completely seals a lung leak and is durable.

Major Task 2: Assess optimal modified alginate gel/patch in a survival surgery model of lung injury in rats.
Subtask 1: Assess survival and animal behavior over the 2 week post-surgical observation period.
Subtask 2: Assess serial chest-rays over the 2 week post-surgical observation period.
Subtask 3: Assess serial blood draws for toxicological evaluations over the 2 week post-surgical observation period.
Subtask 4: Assess lung histology at the end of the 2 week post-surgical observation period.
Milestone(s) Achieved: Demonstration of safety and efficacy of the optimal alginate formulation.

What was accomplished under these goals?

1) Major activities

We have made significant progress in both Major Tasks for Specific Aim 1 and reached the desired milestones. We have now further made significant progress of Major Task 1 in Specific Aim 2. This is described in detail in the below relevant sections. The most promising sealant materials have been refined and, although we have not yet made significant progress on Major Task 2 of Specific Aim 2, this is the subject of future studies and the proposed TTDA application.

2) Specific objectives

The major objective of the proposal is to develop a pleural sealant that will have optimized mechanical and biological properties, coupled with low cost, ease-of use, appropriate storage, and other logistical considerations. Based on promising preliminary data at the time of submission, the proposal was initially focused on methacrylated alginates (AA-MA). Continued study of the AA-MA formulations has defined strengths but also limitations on their use and has stimulated expansion of study into a range of additional biologic materials and other chemical modifications that have resulted in a series of compounds that appear to be more potent as sealants using the *ex vivo* lung models as well as in the pre-clinical (rat) non-survival surgery model. Progress made since the interim Technial/Progress report of January 2017 is detailed below.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

Specific Aim 1
Major Task 1: Develop chemically modified alginate (AA-MA) hydrogels and characterize material properties.
Subtask 1: Synthesize and chemically characterize AA-MA polymer formulations.
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Milestone(s) Achieved: AA-MA hydrogel sealant will exhibit burst pressures beyond the physiological range and will remain adhered to underlying substrate/tissue up to burst pressure.

The result of the studies in **Specific Aim 1** were presented in a technical report submitted 02/14/17**.** Additional developments have been made in regard to Major Task 2 due to advances in the sealant materials and testing. Using a dynamic burst pressure device (**Figure 1**) pressurized by a programmable infuse withdraw syringe pump (kdScientific Legato Series 270p, Holliston MA USA) coupled to a previously described pressure head, sealants were rapidly screened. Burst pressure was recorded using a digital pressure transducer (Omega PX409-015GUSBH, Norwalk CT USA). This system was used for in-house burst pressure testing as it offered advantages in cost and customizability. Burst pressure testing on this new device was validated and proved to be consistent with previously reported data (**Figure 2)**. This is a significant advance in burst pressure device testing and is one we are looking into possible IP.

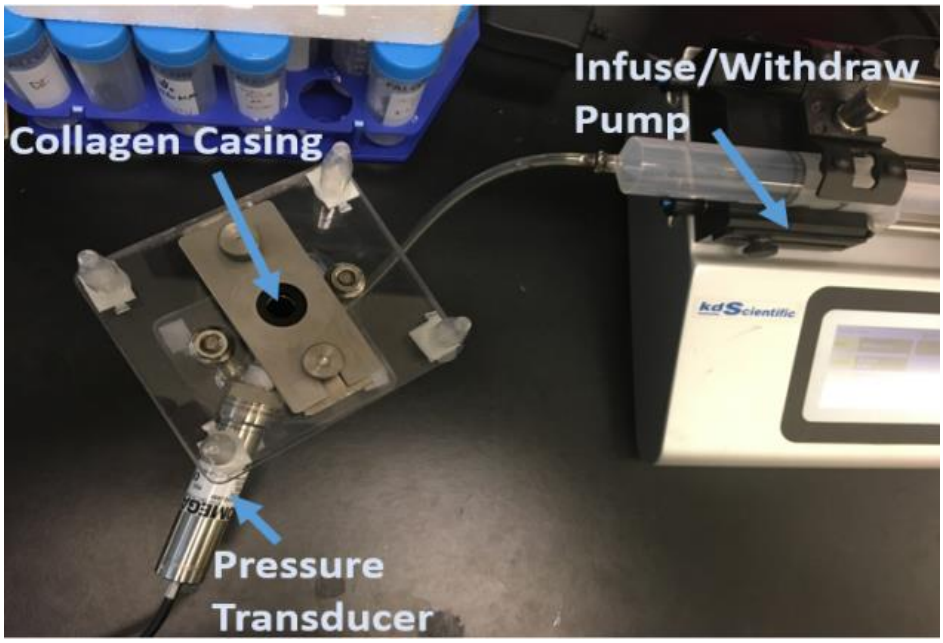

Figure 1: *(right)* Burst Pressure Testing apparatus. A programmable infuse-withdraw pump is used to pressurize a vessel capped with a collagen casing (pressure head described in previous technical report). Pressure is monitored via a high-speed digital pressure transducer connected to a computer interface.

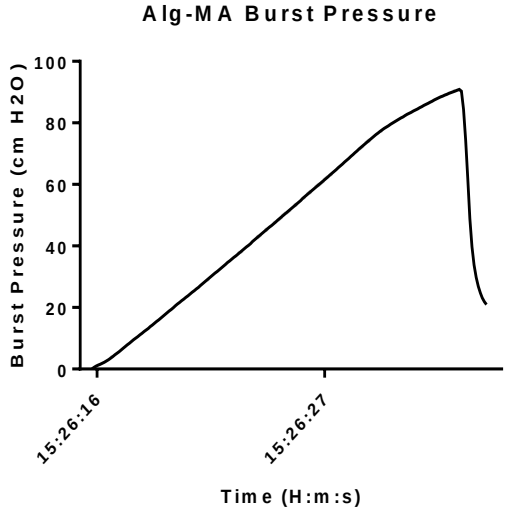

Figure 2: *(left)* Burst pressure testing of 4% methacrylated alginate via a modified burst pressure apparatus. Methacrylated alginate with eosin y based photoinitiator system was extruded onto a collagen burst testing substrate prior to photopolymerization. Following 5 minutes exposure to green light, the polymer was pressurized to material failure. Materials failed due to delamination at a max pressure of 90.9 cmH2O (66.3mmHg), coinciding with materials testing previously reported.

The most promising materials detailed in the interim report were also subjected to burst pressure testing. Blends of methacrylated and cysteine conjugated alginates demonstrated comparable burst pressures to blends of oxidized and methacrylated alginates (**Figure 3)**. Dopamine conjugated alginates (Alg-DA) achieved the highest burst pressures, which exceed physiologic lung pressures (**Figure 4**). This material exceeds the reported maximum strenght of the only FDA approved lung sealant, Progel (Max Alg-DA Burst Pressure=184mmHg;Max Progel Burst Pressure=160mmHg).

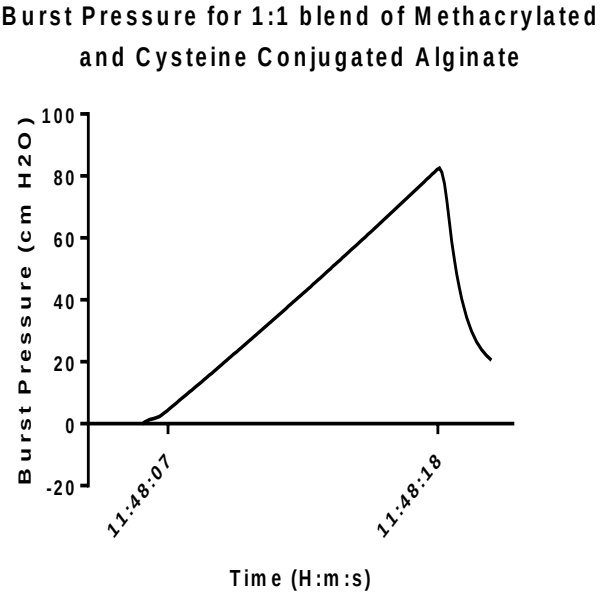

Figure 3: *(left)* Representative figure for burst pressure testing of a 1:1 blend of 3% methacrylated alginate and 3% cysteine conjugated alginate. The polymer blend was extruded onto a collagen burst testing substrate prior to photopolymerization with the eosin y photosystem. Following 5 minutes exposure to green light, the polymer was pressurized to material failure. The maximum burst pressure for this blend was 82.6 cm H2O (60.8 mmHg).

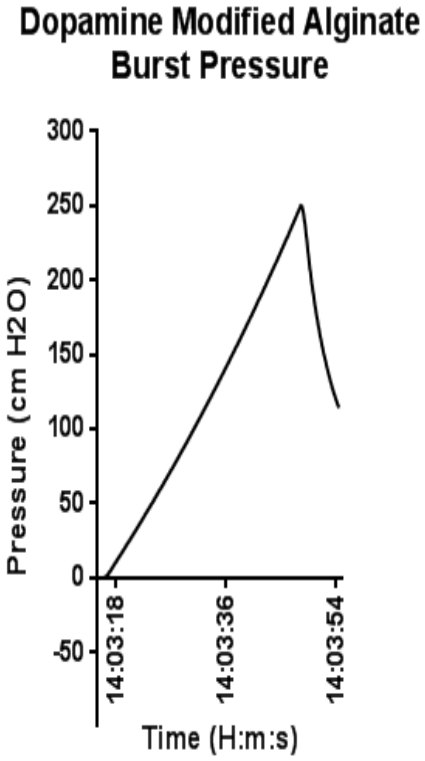

Figure 4: *(left)* Representative figure of the maximum burst pressure of a dopamine conjugated alginate pleural sealant. Dopamine alginate (4%w/v) was extruded onto a collagen substrate with a 2mm defect in the presence of sodium periodate (0.5% w/v, pH 7) and allowed to oxidize for 3 minutes. A maximum pressure of 250.13 cm H2O (184mmHg) was achieved before the material ruptured and began to leak.

Furthermore, to better assess the ability of sealant materials to adhere to tissue proteins in a controlled and high throughput manner, burst pressure testing was optimized. To provide more physiologic relevance, the burst pressure device's collagen substrate was replaced with a decellularized porcine pleural matrix. This decellularized matrix offers specific proteins and the dynamic elasticity of the lung surface, both of which collagen testing substrates lack. Testing on pleural substrates demonstrated better consistency between sealant burst pressure and sealant persistence in rat and pig ex-vivo lung testing models as well as rat in vivo non-survival surgeries. Due to the tissue matched mechanical properties of these pleural matrices, their inherent mechanical strength (**Figure 5**), and the ability of sealants to adhere to pleural proteins, these matrices may also be used to reinforce the sealant materials and have shown promise in non-survival surgery applications.

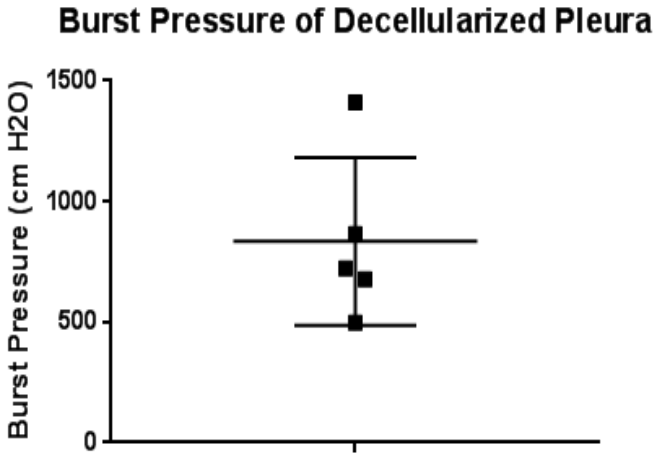

Figure 5: *(left)* Burst Pressure of Decellularized pleural matrices. Pleural matrices are affixed between the rubber plates of the burst pressure device and subsequently pressurized until the matrix bursts. These substrates can be used as a physiologically relevant burst testing substrate, as a buttressing material for hydrogel sealants, or functionalized with catechol bearing motifs to provide adhesive qualities.

These materials were further characterized with an *ex vivo* porcine lung injury model. Pig lungs obtained from a local slaughterhouse were ventilated at physiological parameters in a biosafety cabinet. A 1 cm incision was made in the pleural surface and was then patched using various materials and different methods of application. Patch failure was defined as leakage of air from the lung injury, seen as bubbling from the submerged and ventilated lung. The following is a summary of our data from these experiments.

Table 7: Update on *ex vivo* porcine lung injury model patch testing

	3/24/17							ventilation		
Nr	Materials	Method of Application	Volume (uL)	Crosslinking Method	Crosslinking Time (min)	Time Before Failure (h)	Mode of Failure	PIP (cmH2O)	Frequency (breaths/min)	PEEP (cmH2O)
1	4% HPMC, 4% Alg-SH	dual extruder	1 mL	photo-crosslinking	5	instant	dissolved	20	15	5
2	4% Alg-MA, 4% Alg-SH	dual extruder	1 mL	photo-crosslinking	5	instant	material failure	20	15	5
3	4% Alg-DA, Sodium Periodate	dual extruder	1 mL	Oxidation	1	1 hr	material failure	20	15	5

	4/3/17							ventilation		
Nr	Materials	Method of Application	Volume (uL)	Crosslinking Method	Crosslinking Time (min)	Time Before Failure (h)	Mode of Failure	PIP (cmH2O)	Frequency (breaths/min)	PEEP (cmH2O)
1	4% Alg-DA	dried patch	1 mL	oxidation	1	instant	material failure	20	15	5
2	Alg-SH, Chitosan-PCA	dried patch	1 mL	oxidation	1	instant	material failure	20	15	5

	4/28/17							ventilation		
Nr	Materials	Method of Application	Volume (uL)	Crosslinking Method	Crosslinking Time (min)	Time Before Failure (h)	Mode of Failure	PIP (cmH2O)	Frequency (breaths/min)	PEEP (cmH2O)
1	8% Alg-DA, oxidant	dual extruded	1 mL	oxidation	1	72 hrs	experiment aborted	20	15	5

Summary: Specific Aim 1

Specific Aim 1(specified in proposal) **To optimize the modified alginate for use as a pleural sealant**
Major Task 1: Develop chemically modified alginate (AA-MA) hydrogels and characterize material properties.
Subtask 1: Synthesize and chemically characterize AA-MA polymer formulations.
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Subtask 1: Measure burst pressure and analyze cohesion and adhesion of AA-MA hydrogels on collagen substrates.
Subtask 2: Synthesize AA-MA hydrogels with the ability to covalently link to tissue proteins or create cell-material linkages.
Milestone(s) Achieved: AA-MA hydrogel sealant will exhibit burst pressures beyond the physiological range and will remain adhered to underlying substrate/tissue up to burst pressure.

All milestones were met for **Specific Aim 1** although there is continued room for improvement in material synthesis and mode of application. To this end, as discussed previously and demonstrated above, we have embarked on a systematic exploration of additional biologic compounds and other functional modifications that have produced even more promising results in materials testing and in *ex vivo* lung model evaluations.

Specific Aim 2: To assess the use of optimized modified alginates in an *in vivo* rat lung injury model

Major Task 1: Assess different modified alginate hydrogels and patches in an open-chest in vivo rat model.
Subtask 1: Assess different alginate formulations in the non-survival rat surgery model: evaluation of lung mechanics.
Subtask 2: Assess different alginate formulations in the non-survival rat surgery model: histologic evaluation of lung tissues.
Milestone(s) Achieved: An elastic AA-MA hydrogel will be fabricated which completely seals a lung leak and is durable.

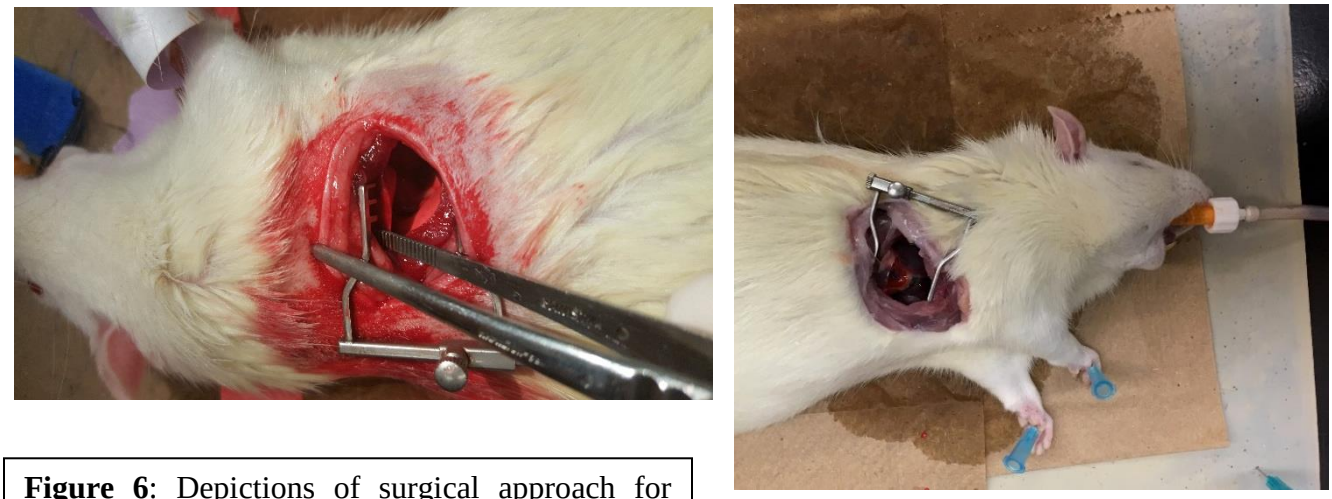

Figure 6: Depictions of surgical approach for non-survival and survival surgery assessments of the pleural sealants.

Non-survival surgery results with new compounds

Further non-survival surgeries have been conducted to test the refined sealant materials (**Figures 6,7**). Previous experiments are outlined in the technical update submitted on 02/14/17. All procedures are performed under a UVM IACUC and a DOD ACURO-approved protocol by appropriately trained laboratory personnel. **Table 8** depicts the experimental approach. Anesthetized rats undergo tracheal cutdown and cannulation and are mechanically ventilated for a 2 hour period. Once the animal is stabilized on the ventilator under appropriate anesthesia, an incision is made in one lung lobe with resulting air leak. The sealant under study is applied and the animal observed for the remainder of the 2 hours period for development of air leaks and for potential failure (material or adhesion) of the sealant.

Figure 7: *(left)* A dopamine alginate patch reinforced by decellularized porcine pleural matrix remains adherent under tension to a cadaver rat lung. The patch stopped a 3mm wound from leaking air.

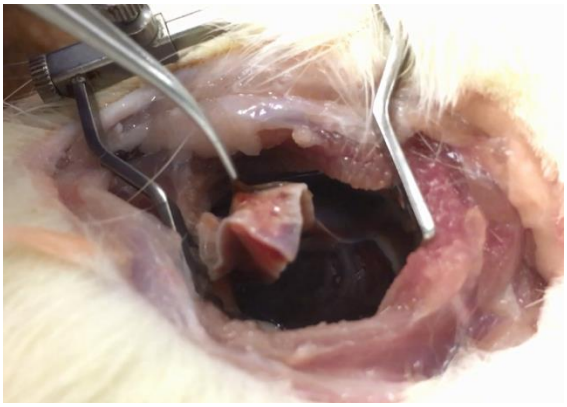

Table 8: Summary of initial in vivo non-survival surgery evaluations

12/16/2016				Application						Ventilation			
Nr	Material	Application Method	Defect Size (mm)	Oxidant	Volume (ul)	Crosslinking Time (min)	Time Before Failure	Mode of Failure	Rat weight (g)	Tidal Volume (mL/100g)	Paralysis	Frequency	PEEP (cmH2O)
1	1:1 Alg-DA:Alg-MA	dried patch	2	Sodium Meta-periodate	100	1	1 min	material failure	500	0.29	no	100	10
2	Chitosan-PCA	dried patch	2	Sodium Meta-periodate	100	1	1 min	terial failu	495	0.29	no	100	10
3	Alg-DA	dried patch	2	Sodium Meta-periodate	100	1	instant	terial failu	485	0.29	no	100	10

4/13/17				Application						Ventilation			
Nr	Material	Application Method	Defect Size (mm)	Crosslinking Method	Volume (ul)	Crosslinking Time (min)	Time Before Failure	Mode of Failure	Rat weight (g)	Tidal Volume (mL)	Paralysis	Frequency	PEEP (cmH2O)
1	8% Alg-DA, oxidant	dual extruded	2	oxidation	500 uL	1	2 hr	experiment aborted	350	3	no	60	0
2	8% Alg-DA, 8% Alg-MA	dual extruded	2	photo	500 uL	5	1 min	material failure	350	3	no	60	0
3	8% Alg-MA, 10% Alg-SH	dual extruded	2	oxidation	500 uL	1	instant	inadequate coverage	350	3	no	60	0

5/17/17				Application						Ventilation			
Nr	Material	Application Method	Defect Size (mm)	Crosslinking Method	Volume (ul)	Crosslinking Time (min)	Time Before Failure	Mode of Failure	Rat weight (g)	Tidal Volume (mL)	Paralysis	Frequency	PEEP (cmH2O)
1	4% Alg-SH, 4% Alg-MA	dried patch	2	photo	200 uL	5	2 hr	experiment aborted	400	3	no	60	0

Major Task 2: Assess optimal modified alginate gel/patch in a survival surgery model of lung injury in rats.
Subtask 1: Assess survival and animal behavior over the 2 week post-surgical observation period.
Subtask 2: Assess serial chest-rays over the 2 week post-surgical observation period.
Subtask 3: Assess serial blood draws for toxicological evaluations over the 2 week post-surgical observation period.
Subtask 4: Assess lung histology at the end of the 2 week post-surgical observation period.
Milestone(s) Achieved: Demonstration of safety and efficacy of the optimal alginate formulation.

Summary of survival surgeries with new materials

An initial pilot experiment was completed to begin our cohort of rat survival surgeries testing our new patch materials. Due to surgical and anesthesia complications these rats expired shortly after extubation following completion of the procedure. In one rat, on autopsy the patch was noted to be intact, while in the other, the patch was leaking. From this initial pilot study we gained critical data and experience that will allow us to refine our surgical procedure and technique for the next set of survival surgeries in future studies that will be the subject of a TTDA proposal.

Table 9: Summary of initial in vivo survival surgery evaluations

	6/14/17			Application						Ventilation			
Nr	Material	Application Method	Defect Size (mm)	Crosslinking Method	Volume (ul)	Crosslinking Time (min)	Surgical Complications	Post-op Course	CT Imaging	Tidal Volume (mL)	Paralysis	Frequency	PEEP (cmH2O)
1	4% Alg-DA, decell pleura	dried patch	2	oxidation	200	1	? Excess anesthetic given	expired 1 minute post-extubation		3	no	40	0
2	4% Alg-DA, decell pleura	dried patch	2	oxidation	200	1	intrathoracic hemorrhage	expired 1 minute post-extubation		3	no	60	0

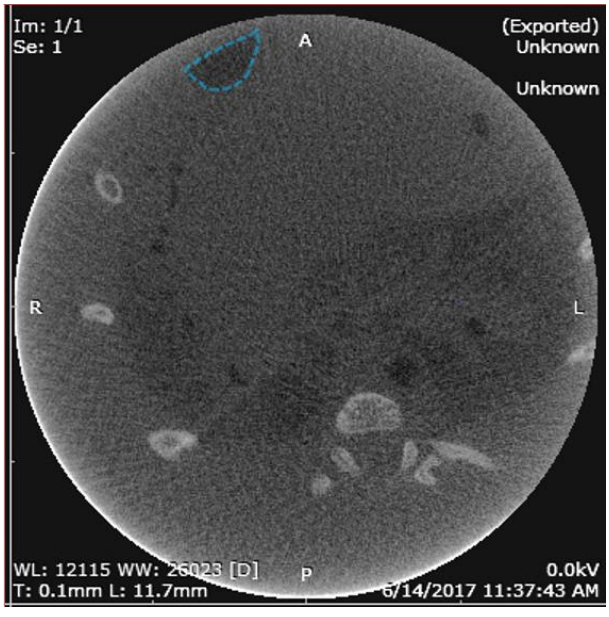

Figure: *(above)* CT scans of rat pneumothorax. Pneumothorax (outlined in blue) was induced by injected 3mL of air into a cadaver rat's thoracic cavity. Red borders outline the rat's lungs. This noninvasive technique will be utilized in future studies to evaluate function of the sealants over time.

Summary of Studies in Specific Aim 2

Strong progress has been made in the non-survival surgeries and we are continuing to use this approach to systematically work through the candidate sealants. After an initial learning curve, the survival surgeries are poised to begin and this approach will comparably be utilized systematically work through the candidate sealants. Future studies will expand the non-survival and survival surgeries to a pig lung model and then to *ex vivo* human lung preparations. These will be part of a TTDA proposal to be submitted in anticipation of engaging with the FDA about potential clinical trials.

References

1) Charron PN, Fenn SL, Poniz A, Oldinski RA. Mechanical properties and failure analysis of visible light crosslinked alginate-based tissue sealants. J Mech Behav Biomed Mater. 2016 Jun;59:314-21.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

As detailed above and in the **Other achievements** section, we have made significant advances in the range of materials being evaluated. This now includes a wider range of biologic materials, including chitosan, hyaluraonate, gelatin, and others coupled with a wider spectrum of functional modifications and functional groups as detailed in the below table. Once a comprehensive evaluation of these new compounds and new approaches has been performed, results will be submitted for peer-reviewed publication and also for presentation at relevant national/international meetings.

4) Other achievements. Include a discussion of stated goals not met.

Stated goals not met

Although we have made significant progress and met milestones for Specific Aim 1, optimization of several sealant formulation that met all stated goals/milestones of **Specific Aims 2 and 3** is still in progress.

Other achievements

We have also continued to expand the scope of original studies to include aerosol application of proposed sealant materials to the external pleural surface and also endoscopic (bronchoscopic) administration directly to the airways. Relevant initial techniques and data were presented in the Technical/Progress report of 2-14-17 and will be the subject of ongoing studies.

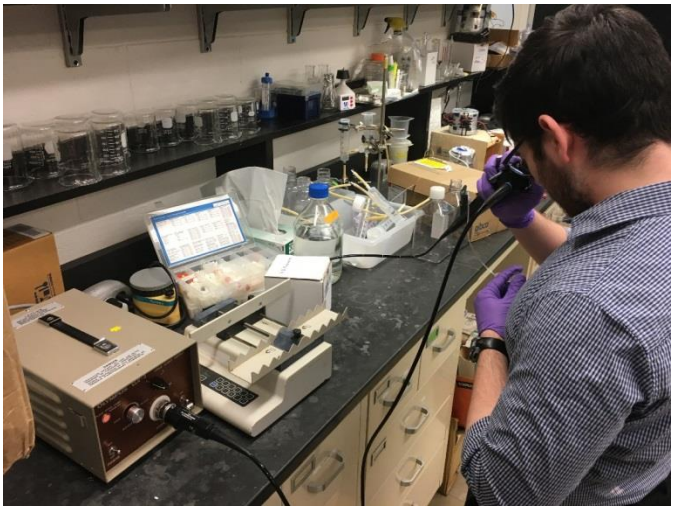

Figure 20: Experimental set-up for endobronchial administration of sealants in the *ex vivo* pig lung model

4) Impact

What was the impact on the development of the principal discipline(s) of the project?

We have made significant and promising progress in the overall goal of developing an effective pleural sealant. This involves developing new investigatory techniques, use of novel materials, and exploration of different application methods. We are optimistic that these will result in a clinically applicable product that can be further investigated in clinical trials.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5) Changes/Problems

Changes in approach and reasons for change

Two significant changes occurred:

1) Change in personnel with removal of previous co-investigator Rachael Oldinski and associated technician Patrick Charron and replacement of services to have been rendered with a subcontract to biomaterials company Akina Inc.. This was discussed with and approved by the DOD on 5-12-16.

2) Expanding scope of investigations to incorporate additional materials, functional modifications, and experimental approaches. As detailed above, these are all logical extensions of the original proposal and remain completely within the spirit and scope of the proposal.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing significant to report

Changes that had a significant impact on expenditures

As above, Akina Inc. was added as a subcontractor in palce of original co-investigator Oldinski. This was a positive change and allowed more extensive and timely development and testing of materials for less overall expenditure. As such, a portion of the grant funds remained unused at the end of the original grant period and was approved by the DOD on 1-17-17 for carry forward for a 6 month period.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6) Products

Nothing to report as yet under any category

7) Participants and Other Collaborating Organizations

Name: Daniel J. Weiss MD PhD
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked:
Contribution to Project: design, implementation, analyses, reporting
Funding Support: DOD

Name: Racheal Oldinski
Project Role: co-investigator. Removed from project as of 5-16-16
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked:
Contribution to Project: original proposal development
Funding Support: n/a

Name: Patrick Charron MS
Project Role: technician. Removed from the project as of 5-16-16
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked:
Contribution to Project: helped develop initial preliminary data
Funding Support: n/a

Name: Franciska Uhl PhD
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked:
Contribution to Project:
Funding Support:

Name: Jacob Dearborn BA
Project Role: Scientist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked:
Contribution to Project:
Funding Support:

Name: Nathan Gasek BA
Project Role: Scientist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project:
Funding Support:

Name: Alexander Riveron MD
Project Role: Scientist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 8
Contribution to Project: Ex Vivo testing and Animal Surgeries
Funding Support:

Name: Zachary Phillips MD
Project Role: Scientist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 8
Contribution to Project: Ex Vivo testing and Animal Surgeries
Funding Support:

Name: John Garner
Project Role: Subcontractor Manager
Researcher Identifier (e.g. ORCID ID): 0000-0002-8024-5061
Nearest person month worked: 1
Contribution to Project: Manager of partner group Akina, Inc. supported synthesis of components and bioadhesion/burst testing.
Funding Support: NA

Name: Faye Jessmon
Project Role: Contract Specialist
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1
Contribution to Project: Technician, supported synthesis and testing at Akina, Inc.
Funding Support: NA

Name: Justin Hadar
Project Role: Scientist
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1
Contribution to Project: Scientist, supported synthesis and testing at Akina, Inc.
Funding Support: NA

Name: Sarah Skidmore
Project Role: Scientist
Researcher Identifier (e.g. ORCID ID): 0000-0003-3321-9634
Nearest person month worked: 1
Contribution to Project: Scientist, supported synthesis and testing at Akina, Inc.
Funding Support: NA

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
The PI, Dr. Daniel Weiss has received several additional grants since the last interim report (Quad Chart of 5-22-16). None are relevant for the current DOD proposal. No new grant funding since the Progress/Technical report of 2-14-17.

1) **NIH R21** (DJ Weiss, P Lee, Co-PIs) 04/01/2017-03/31/2019 1.2 calendar
Decellularized Avian Lungs for Use in Pulmonary Therapeutics
The major goals of this project are to full characterize de-and recellularization of representative avian lungs and to develop initial technologies for novel Avian Lung Assist Devices (ALAD) incorporating recellularized avian lungs that could be potentially utilized for independent, portable, or implantable lung assist devices.

2) **Cystic Fibrosis Research Grant** (DJ Weiss, PI) 11/1/2016-10/31/2018 0.90 calendar

Mechanisms of MSC Actions that Ameliorate Bacterial Lung Infections in CF
The major goal of this project is to further understand the mechanisms by which the mesenchymal stromal cells (MSCs) may be beneficial in treating bacterial infections in lungs of CF patients and use this to maximize any potential therapeutic approaches.

One grant has run its course
1) **Vermont Cancer Center Pilot Award** (Weiss, PI) 6/15/2015-6/14/2016 <0.6 calendar

Development of novel 3D bioprinted scaffolds for reconstructive use in breast cancer patients
The goal of this project is to perform initial materials studies for use in 3D bioprinting of customized breast implants for mastectomy patients.

What other organizations were involved as partners?
Akina Inc. (West Lafayette IN) added as a subcontractor as approved by the DOD on 5-16-16.

Organization Name: Akina Inc.

Location of Organization: West Lafayette IN

Partner's contribution to the project (identify one or more)

Financial support: N/A

In-kind support: Akina manufactures and tests materials used for evaluation as pleural sealants

Facilities: Akina Inc. facilities are utilized for manufacture and materials evaluations of compounds to be tested at UVM in lung injury models

Collaboration: Akina Inc. personnel, led by John Garner, work closely and extensively with Dr. Weiss and his team at UVM

Personnel exchanges: N/A

Other: Akina in collaboration with Dr. Weiss and his team at UVM has submitted an STTR application to the NIH based on work performed under the auspice of the current DOD grant. Further applications by Dr. Weiss and Akina Inc to the DOD will be pursued.

8) Special Reporting Requirements

Collaborative awards: N/A

Quad Charts: see below

PR141815 - "Development of a Novel Alginate-Based Pleural Sealant"
PI: Daniel J. Weiss MD PhD University of Vermont College of Medicine
Budget: $200,000 Topic Area: Respiratory Health Mechanism: Medical Discovery Award

Research Area(s): Respiratory Health: Pleural Sealants Award Status: Open; POP:

Study Goals:

The overall goal is to develop a novel, effective, and easy to use modified alginate-based pleural sealant for use in traumatic and other lung injuries

Specific Aims:

(1) To optimize the modified alginate for use as a pleural sealant
(2) To assess the use of optimized modified alginates in an *in vivo* rat lung injury model

Key Accomplishments:

1. Continued systematic evaluation of modified alginates and other biologic compounds in *in vitro* testing
   A) Systematic synthesis of compounds with different forms of chemical modifications
   B) Systematic materials testing of modified alginate formulations
      1. Burst pressure/adhesion: new improved burst pressure approaches
      2. Gelation/viscosity
2. Continued systematic evaluation of modified alginates in *ex vivo* rodent and pig lung models
3. Initial testing of promising compounds in an *in vivo* non-survival surgery rat lung model
4. Development of novel application methods: external aerosol spraying, endobronchial (bronchoscopic)

Key Outcomes:

1. Progress towards identification of optimized formulations for use in *in vivo* test models

9) Appendices: N/A